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硕 士 学 位 论 文

三疣梭子蟹促性腺激素释放激素及其受体的免疫组化研究

Immunohistochemical Study of Gonadotropin-Releasing
Hormone (GnRH) and Its Receptor of the Swimming Crab,
Portunus trituberculatus

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中英文缩略词对照表

AcN	副叶 (accessory lobe)
AMPN	前脑中部的前端神经髓质 (anterior medial protocerebral neuropil)
AnN	第二触角神经髓质 (antenna II neuropil)
CB	中央体 (central body)
DAB	3, 3-二氨基联苯胺四盐酸盐 (3, 3-diaminobenzidine terehydrochloride)
FSH	卵泡刺激素 (follicle stimulating hormone)
GIH	性腺抑制激素 (gonad inhibitory hormone)
GnRH	促性腺激素释放激素 (gonadotropin-releasing hormone)
GSH	性腺刺激激素 (gonad stimulating hormone)
GtH	促性腺激素 (gonadotrophic hormone; gonadotropin)
LH	黄体生成素 (luteinizing hormone)
LAN	第一触角侧神经髓质 (lateral antenna I neuropil)
MAN	第一触角中部神经髓质 (median antenna I neuropil)
OGTN	嗅球束神经髓质 (olfactory glofactory tract neuropil)
ON	嗅叶 (olfactory lobe)
PB	前脑桥 (protocerebral bridge)
PMPN	前脑中部的后端神经髓质 (posterior medial protocerebral neuropil)
SABC	链霉亲和素-生物素-酶复合物 (strept avidin-biotin complex)
TN	表皮神经髓质 (tegumentary neuropil)

摘要

脊椎动物促性腺激素释放激素（GnRH）由下丘脑分泌，能使腺垂体释放促性腺激素（GtH），是一种非常重要的神经生殖调控因子。近年来的研究显示，GnRH在下丘脑-垂体-性腺调控轴之外也广泛存在并发挥重要作用。GnRH对哺乳动物生殖调控作用的发挥需要其与脑垂体中高特异性的GnRH受体相结合。目前有关甲壳动物GnRH及其受体的研究尚少。

三疣梭子蟹（*Portunus trituberculatus*），隶属于节肢动物门（Arthropoda）、甲壳纲（Crustacea），具有重要的经济价值。本研究在组织学观察的基础上，应用免疫组织化学方法，对三疣梭子蟹神经器官与性腺中 GnRH 及其受体进行了定位研究。

组织学观察发现，三疣梭子蟹的中央神经系统由视神经节、脑神经节和胸神经团组成，具有甲壳动物十足目的典型特征。三疣梭子蟹的视神经节由视神经层、视外髓、视内髓和视端髓组成；脑神经节由前脑、中脑和后脑组成，包括 11 个神经髓质和 12 个细胞群；胸神经团高度愈合，由食道下神经节、胸神经节和腹神经节组成。三疣梭子蟹具有短尾部典型的神经系统，体现了十足目甲壳动物最高等类型的特点。

采用本实验室自行制备的四种不同来源的抗体（章鱼GnRH抗体、海鞘GnRH-I抗体、七鳃鳗GnRH-I和七鳃鳗GnRH-III抗体），应用高灵敏性的SABC免疫组织化学方法，对三疣梭子蟹的神经器官和性腺进行了免疫组织化学定位。结果显示：GnRH的免疫阳性物质广泛存在于三疣梭子蟹神经器官和性腺中。使用不同GnRH抗体所得到的免疫阳性物质分布情况有明显的区别。使用七鳃鳗GnRH-III所得到的免疫阳性反应背景最为清晰、阳性最为强烈。不同发育期三疣梭子蟹神经器官GnRH的免疫阳性分布位置相似，但免疫阳性强度存在一定的差异。GnRH的免疫阳性髓质和免疫阳性神经细胞，主要分布在视神经节的视神经层和X器，脑神经节的前脑和后脑，胸神经团的食道下神经节、胸神经节和腹神经节，性腺的精原细胞、精子细胞、滤泡细胞和卵母细胞等部位。免疫阳性神经细胞的免疫阳性物质均定位在细胞质中。GnRH的免疫阳性物质出现在神经器官的多个部位，说明三疣梭子蟹的相应部位可以合成或分泌GnRH，可能反映了

GnRH作为神经激素起到广泛的调控作用。采用不同来源GnRH抗体所得的免疫阳性物质分布有所不同，这可能与不同来源GnRH抗体对三疣梭子蟹的特异性识别有关。GnRH免疫阳性强度显著的位置可能是合成和分泌GnRH的核心部位。GnRH可能对三疣梭子蟹的性腺发育和神经内分泌起到重要调控作用。

本研究采用兔抗人来源的GnRH受体的抗体，应用高灵敏性的MaxVision™免疫组织化学方法，对三疣梭子蟹的神经器官和性腺进行了免疫组织化学定位。结果显示：GnRH受体的免疫阳性物质广泛存在于三疣梭子蟹神经器官中，但是在不同发育期的性腺中均未检测到GnRH受体的免疫阳性物质。不同发育期三疣梭子蟹神经器官GnRH受体的免疫阳性分布位置相似，免疫阳性强度存在一定的差异。GnRH受体的免疫阳性髓质和免疫阳性神经细胞，主要分布在视神经节的视外髓、视内髓、视端髓和X器，脑神经节的前脑和中脑，胸神经团的食道下神经节、胸神经节和腹神经节等部位。免疫阳性神经细胞的免疫阳性物质均定位在细胞质中。GnRH受体的免疫阳性物质出现在三疣梭子蟹神经器官的多个部位，说明三疣梭子蟹的相应部位可以接受GnRH的调控，GnRH可能通过GnRH受体抑制性腺抑制激素（GIH）的分泌和（或）促进性腺刺激激素（GSH）的分泌，从而促进性腺发育。神经器官中GnRH受体免疫阳性强度显著的位置可能是GnRH作用的核心部位。三疣梭子蟹的性腺中可能存在相对独特的GnRH受体结构类型。

三疣梭子蟹GnRH和GnRH受体免疫阳性物质的特异性分布可为GnRH参与调节神经器官和性腺生理活动提供形态学证据，也对深入理解甲壳动物生殖神经内分泌机制具有重要意义。

关键词：三疣梭子蟹；GnRH；GnRH受体；免疫组织化学

Abstract

Gonadotropin-releasing hormone (GnRH) is secreted from hypothalamus and can affect the adenohypophysis secreting gonadotropin (GtH) in vertebrate. GnRH acts as a very important regulator of neuroendocrine and reproduction. Recent researches show that GnRH also widely exists besides the reproductive regulation axis and plays important roles. It is necessary for GnRH to bind with the high affinity GnRH-receptor in the pituitary gland to regulate the mammalian reproductive process. Up to now, few researches about GnRH and its receptor of crustacean have been reported.

The swimming crab, *Portunus trituberculatus*, which belongs to Arthropoda, Crustacea, is of great commercial value. Based on the histological studies, the method of immunohistochemistry was used in this study in order to find out the locations of GnRH and GnRH receptor in the nervous organs and gonads of *P. trituberculatus*.

Histological observations reveal that: the central nervous system of *P. trituberculatus* is composed by the optic ganglion, the cerebral ganglion and the thoracic ganglion mass, and it shows the typical characters of Crustacean Decapoda. The optic ganglion is composed by lamina ganglionaris (LG), medulla externa (ME), medulla interna (MI) and medulla terminalis (MT). The cerebral ganglion is composed by protocerebrum, deutocerebrum and tritocerebrum, containing 11 neuropils and 12 cell clusters. The thoracic ganglion mass, which is highly coalesced, is composed by suboesophageal ganglion, thoracic ganglion and abdominal ganglion. The central nervous system of *P. trituberculatus* shows the typical characters of Brachyura, reflecting that the Decapoda is the most advanced type in crustacean.

Four different kinds of GnRH antibodies, which are named as octopus GnRH antibody, tunicate GnRH-I antibody, lamprey GnRH-I antibody and lamprey GnRH-III antibody respectively, and were prepared in our laboratory, were used in this study, in order to make sure the locations of the immunoreactive substances in the nervous organs and gonads of *P. trituberculatus*, by using high sensitive SABC immunohistochemical technique. The results reveal that: GnRH immunoreactive

substances were widely detected in the nervous organs and gonads of *P. trituberculatus*. There were obvious differences about the distributions of GnRH immunoreactive substances by using different types of antibodies. The weakest background and strongest immunoreactive intensity were showed in the results by using lamprey GnRH-III antibody. The distributions of GnRH immunoreactive substances were nearly the same, but there were differentiations in the intensity of the immunoreaction. GnRH immunoreactive neuropils and cells were mainly located in the LG and X organ of the optic ganglion, protocerebrum and tritocerebrum of the cerebral ganglion, suboesophageal ganglion, thoracic ganglion and abdominal ganglion of the thoracic ganglion mass, spermatid, follicle cell and oocyte of the gonads. All the immunoreactive substances of the cells were located in cytoplasm. GnRH immunoreactive substances being located in many parts of the nervous organs of *P. trituberculatus*, suggests that the relevant parts can synthesize and secrete GnRH, and so GnRH may act as a neurohormone to widely regulate neuroendocrine and reproduction. The different distributions of the four kinds of GnRH immunoreactive substances may be relevant to their specific recognitions. The strongly marked locations may be the core places of GnRH synthesized and secreted. GnRH may play a vital role in the gonads development and neuroendocrine of *P. trituberculatus*.

Human GnRH receptor antibody against rabbit were used in this study, in order to make sure the locations of the immunoreactive substances in the nervous organs and the gonads of *P. trituberculatus*, by using the high sensitive MaxVisionTM immunohistochemical technique. The results reveal that: the GnRH receptor immunoreactive substances were widely detected in the nervous organs of *P. trituberculatus*, but none in gonads. The distributions of GnRH receptor immunoreactive substances were nearly the same, and there were differentiations in the intensity of the immunoreaction. GnRH receptor immunoreactive neuropils and cells were mainly located in the ME, MI, MT and X organ of the optic ganglion, protocerebrum and deutocerebrum of the cerebral ganglion, suboesophageal ganglion, thoracic ganglion and abdominal ganglion of the thoracic ganglion mass. All the

immunoreactive substances of the cells were located in cytoplasm. GnRH receptor immunoreactive substances being located in many parts of the nervous organs of *P. trituberculatus*, suggests that the relevant parts can be regulated by GnRH. And so, GnRH may inhibit gonadotropin inhibiting hormone (GIH) to/or increase gonads stimulating hormone, to promote the gonads development by binding with GnRH receptor. The strongly marked locations may be the core places of GnRH playing roles. There might be a relatively unique GnRH receptor type in the gonads of *P. trituberculatus*.

The specific distributions of GnRHs and GnRH receptor of *P. trituberculatus* can provide morphological evidences for GnRH participating in the neuroendocrine regulation of nervous organs and gonads, and is meaningful for further study of the reproductive neuroendocrine mechanism in crustacean.

Key words: *Portunus trituberculatus*; GnRH; GnRH receptor; immunohistochemistry

第一章 绪论

1.1 GnRH 的研究进展

1.1.1 脊椎动物 GnRH 的研究进展

促性腺激素释放激素 (gonadotropin-releasing hormone, GnRH), 最初是在20世纪70年代时从猪 (*Sus scrofa*) 和绵羊 (*Ovis aries*) 这两种哺乳动物中分离出来的^[1, 2], 自此开始, 学者们对其展开了一系列的相关研究。

GnRH是在脊椎动物的生殖调控中起到关键作用的一个酰胺化十肽家族。到目前为止, 至少已有24种类型的GnRH被研究者发现, 其中14种来自脊椎动物, 10种来自无脊椎动物, 并且根据它们最早被分离出来的物种进行命名^[3, 4]。脊椎动物中GnRH的系统分布并不是完全严格按照它们的命名分类^[5, 6], 且在脊椎动物所有的类群中, 一个物种都至少同时存在着两种类型的GnRH。

根据NCBI数据库搜索的结果, 目前共有100个脊椎动物物种GnRH的cDNA序列已被公布: 其中23个物种来自哺乳动物, 55个物种来自硬骨鱼类, 7个物种来自两栖动物, 6个物种来自鸟类, 1个物种来自爬行动物豹纹守宫 (*Eublepharis macularius*), 8个物种来自无颌鱼类七鳃鳗。

GnRH结构与功能具有高度保守性。研究发现, 目前已知的脊椎动物GnRH类型都是十肽, 且所有GnRH都具有焦谷氨酸的氨基端和酰胺化的羧基端 (表1.1)。这些十肽在氨基端和羧基端都具有很高的序列保守性^[7]。这些保守区的分子长度在脊索动物的进化过程中始终保持不变。GnRH分子的保守区域对于多肽的生物活性和构想来说是很重要的, 特别是与受体的结合、防止酶解有关, 是受体介导促性腺激素 (gonadotrophin, GtH) 分泌所必需的。这种必要性在对脊椎动物GnRH类似物活性的研究中已经得到了证实^[8]。GnRH十肽的这种选择性的保守使其在脊索动物的进化过程中保持其功能。

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